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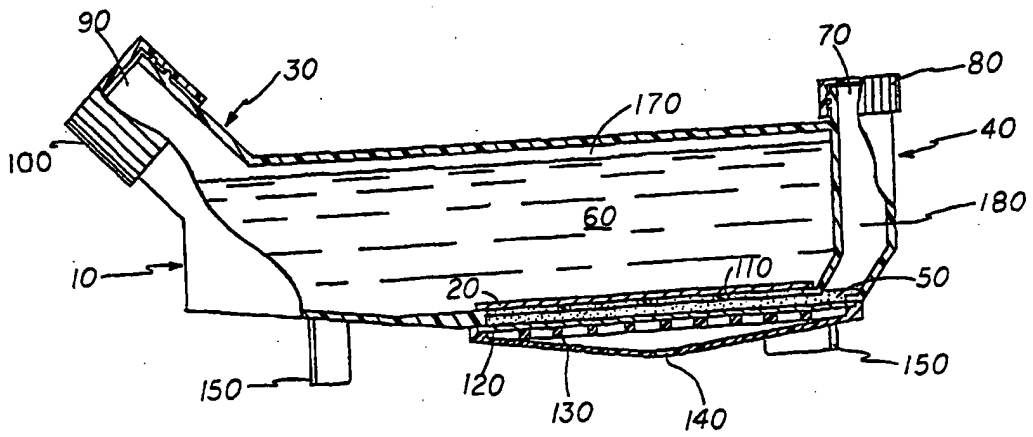

(57) Abstract

A cell culture device (10) comprising a container having a cell culture compartment (40) defined by a lower gas permeable film (120) and an upper sheet (20) selectively permeable to compounds of selected sizes. The device (10) is adapted to allow culture medium (50) to reside between the upper sheet (20) and the lower gas permeable film (120). A basal medium compartment (30) is located above the upper sheet (20) and is adapted to allow basal medium (60) to reside upon the upper sheet (20). Each compartment (30, 40) contains an access port (70, 90). A gas film support (130) below and in partial contact with the gas permeable film (120) holds the gas permeable film (120) in a substantially horizontal position so that suspension or adherent cells can distribute across the surface of the gas permeable film (120).

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COMPARTMENTALIZED TISSUE CULTURE FLASK

BACKGROUND - FIELD OF THE INVENTION

This invention relates to a device and a method for growing cells or tissue in vitro.

BACKGROUND - DESCRIPTION OF PRIOR ART

In vitro growth of mammalian cells is commonly conducted in static culture vessels such as tissue culture flasks (U.S. Pat. No. 3,449,210 issued June 10, 1969 and U.S. Pat. No. 5,151,366 issued Sept. 29, 1992), spinner flasks, and multiple well plate tissue culture plates (U.S. Pat. No. 3,597,326 issued Aug. 3, 1971 and U.S. Pat. No. 4,012,288 issued March 15, 1977). In this type of culture, a portion of the cell culture medium is periodically removed and replaced as cells consume nutrients and produce waste products. This protocol leads to limited cell density, limited cell secreted product concentration, and periodic shifts in nutrient concentration.

Marbrook used a dialysis membrane to separate cells and cell secreted products from the basal medium (Marbrook, J., "Primary Immune Response in Cultures of Spleen Cells", the Lancet, 2, 1279-1281 [1967]). In this device, an inner concentric chamber resides within an outer concentric chamber. The bottom of the inner chamber is comprised of a dialysis membrane which is submerged in basal medium contained in the outer chamber. Cells reside on the membrane receiving nutrients and delivering waste products. Continuous dialysis becomes limited as the membrane loses substrate transport capacity due to the cell mass that resides upon it. Thus, the ability to carry out long term culture is compromised.

Verma (U.S. Patent No. 4,296,205 issued October 20, 1981) teaches of the use of a tissue culture shelf placed in the cell culture compartment to keep cells from directly contacting and clogging the dialysis membrane. The tissue culture shelf has perforations to allow movement of nutrients to the cells. During the culture of suspension cells, the cells and cellular debris are capable of moving through the perforations and coming to rest upon the dialysis membrane, limiting continuous dialysis in long term culture. Also, the architectural structure of the shelf can lead to microenvironments as concentration gradients are unevenly distributed across the surface of the plate.

Vogler (U.S. Patent No. 4,748,124 issued May 31,1988) describes a cell culture compartment that is defined by a lower gas permeable, liquid impermeable sheet

and an upper dialysis membrane. This configuration keeps the dialysis membrane from clogging as cells do not reside upon it, yet dialysis can become limited by other means. Also, the ability to vary oxygen tension is limited relative to Marbrook and Verma. Furthermore, the surface chemistry of materials used to allow gas transfer are limited and in some cases can be undesirable for protein or cell contact. Finally, the teaching does not lead to high density cell culture relative to traditional static culture methods.

The architecture of Vogler can allow dialysis of the cell compartment to become limited. A major problem can arise as liquid evaporates from the growth chamber. Vapor transmission across gas permeable surfaces can be substantial and the loss of liquid will lead to termination of dialysis as liquid contact with the dialysis membrane ceases. Loss of dialysis will also result from off gassing of cell culture medium. Cell culture medium is typically stored at 4 degrees Celsius. As the medium rises in temperature, the gas carrying capacity is reduced and gas bubbles rise and come in contact with the dialysis membrane.

The gas permeable, liquid impermeable sheet of the cell culture compartment limits options available for controlling pericellular pH and P_{O2}. In the prior configurations of Marbrook and Verma, the oxygen tension could be varied by adjusting the liquid level of the cell culture compartment. The structure and method taught by Vogler require oxygen tension be varied by altering the ambient conditions of the atmosphere surrounding the device.

Oxygen tension is very important to cell viability and protein secretion (Reuveny et al., "Factors Affecting Cell Growth and Monoclonal Antibody Production in Stirred Reactors", Journal of Immunological Methods, 86, 53-59 [1986]). The gas permeability of commercially available liquid impermeable sheets and the impact upon pericellular pH and P_{O2} is described in detail by Jenson et al. (Jenson M.D., Wallach D.F.H., and Sherwood P., "Diffusion in Tissue Cultures on Gas- permeable and Impermeable Supports", J. Theor. Biol. (1976) 56, 443-458). The oxygen demands of various cell types combined with the gas permeability of various gas permeable, liquid impermeable sheets will dictate a specific steady state pericellular pH and P_{O2} for each combination. This means cell lines are subject to very limited pericellular conditions. Creating different pericellular conditions is achieved by altering the ambient conditions of the incubator in which the device resides. As a practical matter, this is difficult for

researchers who maintain incubators at standard conditions for a wide variety of simultaneous uses.

Gas permeable, liquid impermeable sheets also limit the surface chemistry available for support of cells and protein structures. The proliferation and function of many cell types is strongly affected by the chemical nature of the surfaces they reside upon. The surface chemistry of liquid impermeable material is incompatible with many cell types and protein structures. Also, hydrophobic material which is often the basis for gas permeable, liquid impermeable films, can cause non-specific protein binding. This in turn can lead to depletion of soluble growth factors. Thus, further modification to the materials may be required for optimization of the cell environment.

The architecture of Vogler also leads to limited cell density. The growth chamber will deform in shape due to the weight of liquid residing upon it and pressure of fluid expansion, leading to a sagging gas permeable sheet. This allows suspension cells to settle in the low point of the sheet. High localized cell densities at the low point of the sheet leads to excessive resistance to flux of nutrients and a localized reduction in cell viability. Furthermore, the cells are unable to expand to other areas of the gas permeable sheet.

It is accordingly an object of the present invention to provide a method and devices for the long term culture of anchorage dependent cells and suspension cells at high density, simultaneously allowing variable oxygen tension, an even distribution of cells across the bottom of the culture compartment, uninterrupted dialysis, and a wide variety of surface chemistry options. Still further objects and advantages will become apparent from consideration of the ensuing description and drawings.

SUMMARY OF THE INVENTION

Many of the problems of the prior art are solved by compartmentalized cell culture devices constructed in accordance with this invention to allow cells to be cultured at high density over a long period of time.

Specifically, there is provided a cell culture compartment including a lower gas permeable film and spaced vertically therefrom an upper sheet selectively permeable to compounds of selected sizes, means spacing said film from said sheet so as to allow a culture medium to reside between said upper sheet and said lower gas

permeable film. There is a means defining a basal medium compartment to allow a basal medium to reside upon said upper sheet. There are access ports to said cell culture compartment and to said basal medium compartment. There exists a gas film support below and in partial contact with said gas permeable film whereby a major portion of said gas permeable film is held in a substantially horizontal position such that cells can distribute across the horizontal portion of said gas permeable film and gas transfer into and out of said cell culture compartment is not substantially impaired. The gas permeable film can be any biocompatible liquid permeable or impermeable, hydrophobic or hydrophilic, porous or non porous material which provides the appropriate pericellular environment and surface chemistry for a specific cell culture application.

The upper basal medium compartment and the lower cell culture compartment are configured to allow pipette access and prevent pressurization due to temperature increase. The cell culture compartment is configured to prevent loss of dialysis due to evaporation or off-gassing, compensate for liquid flux from the basal medium reservoir, and allow high cell density cultures to be maintained over a long period of time.

According to one feature of the invention, evaporative loss of cell culture medium can be controlled independent of ambient conditions by providing gaseous exchange of the cell culture compartment by way of the humidified gas of the upper basal medium compartment.

According to a second feature of the invention, oxygen tension within the cell culture compartment can be accurately controlled independent of ambient conditions by adding a third compartment that utilizes a variable level of liquid to alter oxygen tension.

According to a third feature of the invention, the cell culture compartment volume can be varied during operation with out interrupting dialysis.

According to a fourth feature of the invention, the transfer of air to the cell culture compartment can be minimized during routine handling to minimize pH fluctuations.

According to another embodiment of the invention, there is provided a plurality of cell culture compartments each including a lower gas permeable film and spaced vertically therefrom an upper sheet selectively permeable to compounds of selected sizes, means spacing said film from said sheet so as to allow a culture medium to reside between said upper sheet and said lower gas permeable film. There is a means defining a basal medium compartment to allow a basal medium to reside upon said upper sheet. There are access ports to each of said cell culture compartments and to said basal medium compartment. There exists a gas film support below and in partial contact with each of said gas permeable films whereby a major portion of each gas permeable film is held in a substantially horizontal position such that suspension cells can distribute across the horizontal portion of said gas permeable film and gas transfer into and out of said cell culture compartment is not substantially impaired.

With these structures, a method of culturing cells at high density becomes available. Also, a method of controlling oxygen tension surrounding cells becomes available by utilizing a liquid barrier to oxygen flux.

With the invention so stated, many of the problems associated with the prior art are solved. Long term high density in vitro culture of both suspension and adherent cells is possible with simultaneous provisions for variable oxygen tension, controlled evaporation, long term maintenance of small cell compartment liquid volumes, and uninterrupted dialysis.

A BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a cut away view taken through the center of a compartmentalized tissue culture flask;

Fig. 2 is a cross-sectional view of a compartmentalized tissue culture flask showing an embodiment using a gas permeable membrane, portions of which project into the cell culture compartment.

Fig. 3 is a cross-sectional view of a compartmentalized tissue culture flask showing an embodiment that balances hydrostatic pressure in two compartments.

Fig. 4 is a cross-sectional view of a compartmentalized tissue culture flask showing an embodiment that controls evaporation; and

Fig. 5 is a cutaway view of a compartmentalized flask that minimizes pH fluctuations during routine handling.

Fig. 6 is a side view of a compartmentalized flask standing on end.

Fig. 7 is a cross-sectional view of compartmentalized tissue culture flask showing an embodiment that allows variable oxygen tension.

Fig. 8 is a top view of a compartmentalized multiple well tissue culture plate in accordance with the present invention;

Fig. 9 is a partial cross-section taken through section A-A of the multiple well tissue culture plate of Fig. 8; and

Fig. 10 is a top view of a compartmentalized tissue culture plate in which basal medium resides in a common reservoir.

Reference Numerals in Drawings

10	compartmentalized tissue culture flask
15	compartmentalized multiple well tissue culture plate
20	membrane
30	basal medium compartment
40	cell culture compartment
50	culture medium
60	basal medium
70	cell culture compartment access port
80	cell culture compartment access port cap
85	top cover
90	basal medium access port
100	basal medium access port cap
110	membrane support
120	gas permeable film

130 gas film support
140 gas access opening
150 feet
170 basal medium head space
180 culture medium head space
190 gas access channel
200 gas access channel cover
210 drain port
220 variable oxygen control compartment
230 lower gas permeable film
240 oxygen control compartment bottom
250 oxygen control compartment access port
260 liquid resistor
270 upper membrane support
280 skirt
290 notches
300 notch cover
310 notch cover hinge

DETAILED DESCRIPTION

Referring now more specifically to the drawings, Fig. 1 shows a cutaway view of the invention in the embodiment of a compartmentalized tissue culture flask 10. A membrane 20 separates compartmentalized tissue culture flask 10 into a basal medium compartment 30 and a cell culture compartment 40. A culture medium 50 containing cells or tissue resides in cell culture compartment 40. A basal medium 60 resides in basal medium compartment 30. Access to cell culture compartment 40 is provided by a cell culture compartment access port 70 which is covered by a cell culture compartment access port cap 80. Access to basal medium compartment 30 is provided by a basal medium access port 90 which is covered by a basal medium access port cap 100. A membrane support 110 stabilizes membrane 20. A gas permeable film 120 resides on top of a gas film support 130 which is adapted to allow gas to contact the vast majority of the surface of gas permeable film 120 by way of a gas access opening 140. Feet 150 lift gas film support 130 above the surface on which compartmentalized tissue culture flask 10 resides.

In operation, culture medium 50 containing cells or tissue of interest is introduced into cell culture compartment 40 through cell culture compartment access port 70 until it makes complete contact with the underside of membrane 20. Basal medium 60 is introduced into basal medium compartment 30 through basal medium access port 90. Preferably, a basal medium head space 170 will be maintained between basal medium 60 and the top of basal medium compartment 30 and basal medium access port cap 100 will be slightly loosened. This allows ambient gas to influence the pH of basal medium 60. A basal medium access port cap of the type used in Falcon® tissue culture flasks (commercially available from Becton Dickinson Labware -- Plymouth, England) may be used in cases where the cap should remain tightened due to contamination concerns.

Compartmentalized tissue culture flask 10 is designed to prevent pressurization as the temperature of the gas and liquid it contains rises when it is placed into an incubator. Cell culture access port cap 80 and basal medium access port cap 100 can be loosened to allow expanding gas to vent to the surrounding atmosphere. Culture medium 50 is free to expand into a culture medium head space 180 and basal medium 60 is free to expand into basal medium head space 170. In this manner pressure does not affect the flatness of gas permeable film 120, or liquid flux through either membrane 20 or gas permeable film 120.

Aside from fluid expansion, another phenomenon occurs as a result of the temperature increase. The gas carrying capacity of the liquid medium is lowered. Gas bubbles that are released by culture medium 50 can be moved to culture medium head space 180 by temporarily tilting compartmentalized tissue culture flask 10. This prevents the gas from becoming trapped against the bottom of membrane 20 and limiting dialysis.

Culture medium head space 180 is designed to counterbalance liquid transfer that may occur through membrane 50 due to hydrostatic pressure differential that results from head height differences in basal medium 60 and culture medium 50 at the time of set up. As liquid moves through membrane 20 into cell culture compartment 40, the level of culture medium in head space 180 rises. If cell culture compartment access port cap 80 is in the tightened position, the liquid will continue to rise in culture medium head space 180 until the hydrostatic pressure of culture medium 50 is balanced by the pressure of the displaced gas. If cell culture compartment access port cap 80 is in the loosened position, liquid will rise in culture medium head space 180 until it reaches a level where the diminished pressure differential across membrane 50 stops liquid transfer.

In the preferred embodiment, the volume of culture medium 50 after flow has stopped will be no more than two times the volume of culture medium 50 at the onset of the culture.

Membrane 20 is a material selectively permeable to a class of molecules. Several types of material are acceptable including cellulose, polyacrylinitrile, polysulfone, polycarbonate, and polyacrilamide. For example, dialysis membranes retaining molecules and compounds with molecular weights greater than 15,000 are commonly used to culture murine hybridoma cells. By using a membrane with this characteristic, cells, growth factors, and secreted antibodies are retained in cell culture compartment 40. In other applications, it may be advantageous to allow larger molecules and compounds to pass freely between basal medium 60 and culture medium 50. For example, high density culture of lymphocytes may require a large quantity of growth stimulating factors to be present. These factors, such as interleukin 2, can be introduced into basal medium 60 and culture medium 50. By appropriately selecting the pore size of membrane 20, a large source of these factors can be made available to the lymphocytes.

Membrane 20 will not exceed a molecular weight cutoff of 150,000 Daltons in most applications. Yet, there are applications where even larger pore sizes may be desirable. For example, if the purpose is only to culture a large number of cells, any pore size which retains the cells in cell culture compartment 40 can be used. In this case, a 0.2uM or 0.45uM microporous polycarbonate membrane such as that commercially available from Poretics Corporation (Livermore, California) could be used.

Membrane support 110 stabilizes membrane 20. As basal medium 60 is added to basal medium compartment 30, the weight is transferred to membrane 20. Membrane support 110 keeps membrane 20 from sagging and displacing culture medium 50 into culture medium head space 180. Membrane support 110 makes minimal contact with membrane 50 so the surface area for dialysis is not substantially diminished. Membrane support 110 is designed such that it will allow gas bubbles to move freely to culture medium head space 180. Membrane support 110 can be made of any biocompatible material. In the preferred embodiment it is clear plastic such as polystyrene or polycarbonate. If membrane 20 is a material cast onto a stiff mesh backing or precise control of the volume of culture medium 50 residing above gas permeable film 120 is not required, membrane support 110 is optional.

The embodiment of Fig. 2 shows a configuration where portions of gas permeable film 120 project into cell culture compartment 40 to provide support of membrane 20 without the need for membrane support 110. Silicone is a good choice for material as it can be readily molded to form an appropriate shape. Wall thickness can be minimized to allow additional gas transfer into cell culture compartment 40. In the case of silicone, average wall thickness should be kept below 0.015 inches, preferably about 0.004 to 0.012 inches.

The embodiment shown in Fig. 3 keeps membrane 20 from sagging and insures liquid maintains contact with the upper and lower surface of membrane 20 during operation. It is particularly useful for applications in which a high concentration of cells is desired. Membrane support 110 is not present and this allows a very small volume of culture medium 50 to be used as well as preventing obstacles to cell removal. This embodiment is also capable of functioning with various volumes of culture medium 50, as liquid contact with membrane 20 is always assured.

In operation, membrane 20 is pressed onto the surface of gas permeable film 120 by the weight of basal medium 60. Putting the membrane in this position can also be achieved by generating a vacuum on cell culture compartment 40. A predetermined volume of culture medium 50 containing the desired culture is then introduced into cell culture compartment 40 by way of cell culture compartment access port 70. When basal medium access port cap 100 and cell culture compartment access port cap 80 are in the vented position, culture medium 50 will come to rest within culture medium head space 180 at a level that counterbalances the hydrostatic pressure of basal medium 60.

In the preferred embodiment, the volume of culture medium 50 residing in culture medium head space 180 will be a small fraction of the volume of culture medium 50 residing between membrane 20 and gas permeable film 120. It is possible for water from basal medium 50 to move into cell culture compartment 40 if severe osmotic gradients develop across membrane 20. If this condition begins to occur, cell culture compartment access port cap 80 should be placed in the tightened position. This will prevent liquid from rising in culture medium head space 180.

Introducing culture medium 50 into cell culture compartment 40 will require enough pressure to overcome the hydrostatic pressure of basal medium 60. This

can be accomplished by configuring cell culture compartment assess port 70 to accept a pipette, syringe, or some other culture medium container such as a bag or bottle. Culture medium 50 can be removed in the same manner.

This method of introducing culture medium 50 into cell culture compartment 40 and removing it therefrom can be utilized in all of the embodiments described herein. In most embodiments using membrane support 110, it will be necessary to provide a vent to allow air access to and from cell culture compartment 40. The vent is required to allow gas to be displaced when culture medium 50 is introduced into culture compartment 40. The vent also allows gas to displace culture medium 50 when it is removed from cell culture compartment 40. Cell culture compartment access port 70 is designed to allow gas to move in and out while culture medium 50 is added and removed. Thus, cell culture compartment 40 is effectively vented.

The embodiment of Fig. 3 does not require a vent. When membrane 20 is pressed against gas permeable film 120, air is displaced from cell culture compartment 40 prior to introducing culture medium 50. When culture medium 50 is removed, membrane 20 is simply lowered. Thus, there is never the need for gas and liquid to move to and from cell culture compartment 40 simultaneously.

When the embodiment shown in Fig. 3 is used for high density culture, the average distance between membrane 20 and gas permeable film 120 should be less than 5 millimeters, preferably about 1 mm to 2 mm.

The embodiment of Fig. 3 can also be used to prevent evaporation of culture medium 50 from allowing membrane 20 to lose contact with culture medium 50. Membrane 20 is essentially floating on culture medium 50 and as culture medium 50 evaporates through gas permeable film 120, membrane 20 simply gets closer to gas permeable film 120. No dialysis limitation occurs, as membrane 20 is always in contact with culture medium 50.

In cases where membrane 20 is comprised of a material such as cellulose that swells or becomes baggy when wet, it may be desirable to constrain membrane 20 with an upper membrane support 270. Upper membrane support 270 stops upward travel of membrane 20 as culture medium 50 enters cell culture compartment 40. Culture

medium 50 presses membrane 20 against upper membrane support 270, smoothing wrinkles.

Wrinkles in membrane 20 lead to an uneven distribution of cells during inoculation. If membrane 20 were severely wrinkled, culture medium 50 would reside within the wrinkles. Then some areas above gas permeable film 120 would have more culture medium 50 residing above it than others. Cells in the inoculum are distributed equally throughout culture medium 50. Eventually, these cells settle onto gas permeable film 120. Area of gas permeable film 120 in which a larger volume of culture medium 50 resides above it will receive more cells. To prevent this condition, the wrinkling of membrane 20 should be minimized.

Upper membrane 270 can be any biocompatible material such as virgin grade polystyrene or polypropylene. Care should be given to insure that it does not limit dialysis. In the preferred embodiment, it should be about 70% to 90% open.

Gas permeable film 120 is a biocompatible material capable of allowing transfer of gas into and out of cell culture compartment 40. Gas permeable film 120 can be either liquid permeable or impermeable, hydrophobic or hydrophilic, porous or non porous. Thickness can range above or below 0.25 mm. The best choice depends on the specific application. As a general guideline, the gas permeability of a given membrane should be considered in addition to the interaction of the membrane with either cells or protein structures. Liquid impermeable films of equivalent thickness will establish various steady state oxygen tension at the cell/film interface. FEP Teflon, silicone, and silicone polycarbonate copolymers will establish higher oxygen tension than polyethylene, polycarbonate, polypropylene, polysulfone. or polypropylene. In applications where protein denaturization, non-specific protein binding, cell membrane damage, or cell attachment is affected by the surface chemistry of the film, hydrophilic surfaces are more suitable. In applications where it is desirable to maintain the entire cell membrane in contact with water, hydrated gels may be most suitable

The use of certain materials not normally associated with gas exchange can expand the options available for controlling oxygen tension at the cell/film interface. For example, non-porous cellulose acetate has a relatively low oxygen gas permeability on the order of 7.3×10^{-9} $cm^3 \cdot cm/(sec \cdot cm^2 \cdot atm)$. When cellulose acetate is made porous, it will increase oxygen permeability as it absorbs culture medium 50 with an

oxygen permeability of 1.4×10^{-6} $cm^3 \cdot cm/(sec \cdot cm^2 \cdot atm)$. In this manner, varying oxygen tension can be achieved by controlling the amount of culture medium 50 present in gas permeable film 120. Thus, oxygen tension variations will result by varying either the pore size, porosity, or tortuosity of gas permeable film 120.

Gas film support 130 holds gas permeable film 120 in a substantially horizontal position and stabilizes gas permeable film 120 to prevent sagging. Care should be given to assure the flatness of gas permeable film is such that cells do not roll into or otherwise collect in low points. This is an undesirable event as the piling up of cells will create diffusional limitations and lead to cell death. On the other hand, care must also be taken to assure that gas exchange remains adequate. Thus, the optimal amount of contact gas film support 130 makes with gas permeable film 120 will depend on the stiffness and gas permeability of gas permeable film 120 as well as gas exchange and metabolic requirements of a particular cell culture application. It should be expected that most cell lines will become diffusionally limited at about ten to fifteen cell layers.

Gas film support 130 also acts to protect gas permeable film 120 from contamination or puncture. Minimal contact with gas permeable film 120 is made to allow the maximum possible surface area for gas transfer. Gas access opening 140 is located at the lowest point of gas film support 130 to allow condensation to exit gas film support 130. It is sized to allow adequate gas exchange of cell culture compartment 40 while minimizing evaporation. Gas film support 130 can be made of any structurally stable material, but in the preferred embodiment is an optically clear material such as polystyrene or polycarbonate to allow visual inspection of the culture in cases where gas permeable film 120 is optically clear. Feet 150 elevate compartmentalized tissue culture flask 10 such that gas film support 130 does not become scratched or visually impaired.

Another consideration with regard to material selection for gas permeable film 120 is the moisture vapor transmission rate. Culture medium 50 will evaporate at various rates pending the material selection of gas permeable film 120. Limiting the cross-sectional area of gas access opening 140 can reduce the rate of evaporation, although the rate of liquid loss will also be a function of the ambient humidity which is more difficult to control. The configuration of Fig. 4 addresses this issue.

The humidified gas of basal medium head space 170 is placed in communication with the underside of gas permeable film 120 by a gas access channel

190. A gas access channel cover 200 prevents basal medium 60 from entering gas access channel 190 and limiting gas transfer. Gas access channel cover 200 is a gas permeable, liquid impermeable film. To prevent condensation from accumulating upon gas access channel cover 200 and diminishing gas transfer, it is not in a horizontal position. Thus. condensation can return to basal medium 60 by gravitational force. Also, gas access channel 190 is capable of collecting condensation in a drain port 210.

Many other methods of placing basal medium head space 170 in communication with gas permeable film 120 are possible. Care should be given to prevent condensation or basal medium 60 from diminishing gas transfer.

The configuration of Fig. 4 can also assist in pH control when the device is removed from the incubator for routine handling. Normally, compartmentalized tissue culture flask 10 resides in a CO_2 incubator. This allows the pericellular pH of cell culture compartment 40 to be maintained at an appropriate level. When compartmentalized tissue culture flask 10 is removed from an incubator and placed into a laminar flow hood for cell handling, the pH of cell culture compartment 40 can fluctuate rapidly as CO_2 is stripped from culture medium 50. The configuration of Fig. 4 allows the CO_2 of basal medium compartment 30 to become accessible to culture medium 50.

Another configuration that addresses this issue is shown in Fig. 5. A skirt 280 surrounds the base of compartmentalized tissue culture flask 10. Skirt 280 severely restricts air access to the underside of gas permeable film 120 when the device resides in a laminar flow hood. Thus, CO_2 is retained in culture medium 50, maintaining the pH level. The shelves of most CO_2 incubators have openings that will allow gas direct access to the underside of gas film support 130. Thus, skirt 280 does not impede gas transfer when the device resides in most incubators. For the case where the incubator does not have perforated shelves, notches 290 can be created in skirt 280. A notch cover 300 covers each of notches 290. Notch cover 300 rotates on notch cover hinge 310 and is placed in the closed position when the device is removed from the incubator and in the open position when the device resides in the incubator. Fig. 5 shows notch cover in the open position. Many other mechanisms for allowing the skirt to be either open to gas access or closed to gas access are possible.

Fig. 6 shows another configuration of compartmentalized tissue culture flask 10 which also limits gas transfer when the device is removed from the incubator. In

this configuration, the device stands on end. Culture medium 50 collects in the lower portion of cell culture compartment 40. The contact area between culture medium 50 and gas permeable film 120 is significantly reduced. Thus, gas transfer is reduced in direct proportion to the reduced contact area. This reduces the movement of CO_2 from culture medium 50 to the ambient environment and delays a shift in pH.

If the type of materials available for gas permeable film 120 do not provide the desired oxygen tension, the configuration shown in Fig. 7 can be utilized. A variable oxygen control compartment 220 is composed of a lower gas permeable film 230 supported in a horizontal position by an oxygen control compartment bottom 240. An oxygen control compartment access port 250 allows a liquid resistor 260 to be introduced into variable oxygen control compartment 220. The oxygen tension at the bottom of gas permeable film 120 can be carefully controlled by varying the height of liquid residing upon a lower gas permeable film 230 in accordance with Fick's Law. Lower gas permeable film 230 can be any highly gas permeable film or sheet. In the preferred embodiment, it is liquid impermeable and has a relatively low moisture vapor transmission rate. Oxygen control compartment bottom 240 allows the vast majority of lower gas permeable film 230 to be in communication with the ambient environment. A hermetic seal exists between lower gas permeable film 230 and oxygen control compartment bottom 240. This seal can be made by welding, adhesives, or any other suitable method. The distance between the top of lower gas permeable film 230 and the bottom of gas permeable film 120 will preferably be about 5 to 20 mm.

To minimize evaporation of liquid residing in variable oxygen control compartment 220, the underside of lower gas permeable film 230 can be placed in gaseous communication with basal medium head space 170 as previously described.

Although there is no restriction on either the shape or size of cell culture compartment 40, the advantageous distance between gas permeable film 120 and membrane 20 is about 1 to 5 millimeters to obtain a high concentration of cells and cell secreted products. When gas permeable film 120 is substantially flat and horizontal, up to 30×10^6 cells per square centimeter of surface area can be expected to remain viable. These cells can pile up to a height of about 300 micrometers. Thus, membrane 20 is in no danger of contacting cells and becoming clogged when it resides at least 1 mm from gas permeable film 120.

In order to minimize the frequency of basal medium 60 exchanges, the volume of basal medium 30 is sized relative to the surface area of gas permeable film 120. For suspension cells that reside in static culture at one million cells per milliliter, about 5 to 10 ml of basal medium 60 are required for every 1 cm^2 of gas permeable film 120. When culturing anchorage dependent cells growing in monolayer, advantageously the volume of basal medium 60 exceeds the surface area of gas permeable film 120 by at least a factor of two.

The housing of compartmentalized culture flask 10 can be any biocompatible material. In the preferred embodiment, the housing will provide optical clarity so the medium can be visually monitored for determining the pH of the medium or detecting possible microbial contamination. Polystyrene is a favored selection. Construction of compartmentalized tissue culture flask 10 can be by ultrasonic welding, mechanical fasteners, solvent bonding or any other method which provides leak proof integrity. Gas permeable film 120 and membrane 20 can be sealed by o-rings, gaskets, welding, adhesives, or any other method which provides leak proof integrity. In the preferred embodiment, all materials used in the compartmentalized tissue culture flask 10 should be compatible with gamma sterilization.

Fig. 8 is a top view of an additional embodiment of this invention in the format of a compartmentalized multiple well tissue culture plate 15. Fig. 9 shows section A-A of Fig. 8. In this configuration, a number of different cultures can conducted in one device. This drawing shows an embodiment that can allow six cultures. The device can constructed to allow more or less cultures if desired. Basal medium 60 resides in basal medium compartment 30. A top cover 85 resides over basal medium compartment 30 and protects basal medium 60 from contaminants.

Fig. 9 is a top view of a further embodiment of a compartmentalized multiple well tissue culture plate 15. In this format basal medium compartment 30 is in communication with more than one cell culture compartment 40.

As described previously in Fig. 2, a configuration where portions of gas permeable film 120 project into cell culture compartment 40 to provide support membrane 20 without the need for membrane support 110 is possible.

As described previously in Fig. 3 , a configuration adapted to include features beneficial for high density cell culture and variable control over the volume of culture medium 50 is possible.

As described previously in Fig. 4 , a configuration that incorporates features for placing the humidified gas of the basal medium compartment 30 in communication with the underside of gas permeable film 120 is possible.

As described previously in Fig. 5 , a compartmentalized multiple well tissue culture plate 15 can be adapted to include skirt 280 to prevent rapid pH changes when the device is removed from a CO_2 incubator, for routine handling, as previously described.

As described previously in Fig. 7 , an embodiment configured to include the features of variable oxygen control compartment 220 is possible.

Those skilled in the art will appreciate that numerous modifications can be made thereof without departing from the spirit. Therefore, it is not intended to limit the breadth of the invention to the embodiments illustrated and described. Rather, the scope of the invention is to be determined by the appended claims and their equivalents.

What is claimed is:

1. A cell culture device comprising a container having

a) a cell culture compartment 40 defined by a lower gas permeable film 120 and an upper sheet 20 selectively permeable to compounds of selected sizes and adapted to allow a culture medium to reside between said upper sheet 20 and said lower gas permeable film 120;

b) a basal medium compartment 30, at least a portion of which is above said upper sheet 20, and adapted to allow a basal medium 60 to reside upon said upper sheet 20;

c) an access port 70 to said cell culture compartment 40;

d) an access port 90 to said basal medium compartment 30; and

e) a gas film support 130 below and in partial contact with said gas permeable film 120 whereby the majority of said gas permeable film 120 is held in a substantially horizontal position such that suspension cells can distribute across the horizontal portion of said gas permeable film 120 and gas transfer into and out of said cell culture compartment 40 is not substantially impaired.

2. A device according to claim 1 wherein the surface area of said upper sheet 20 is at least one quarter of the surface area of said lower gas permeable film 120.

3. A device according to claim 1 wherein the average distance between the substantially horizontal portion of said lower gas permeable film 120 and said upper sheet 20 is less than 15 millimeters.

4. A device according to claim 1 wherein said gas film support 130 is adapted such that the smallest cross-sectional area of said gas film support 130 open to gaseous communication with the ambient environment is less than the total surface area of said lower gas permeable film 120 in contact with gas.

5. A device according to claim 1 wherein an upper sheet support 110 resides below and in partial contact with said upper sheet 20.

6. A device according to claim 1 wherein at least a portion of the entrance of said access port 90 to said basal medium compartment 30 is positioned higher than the top of said basal medium compartment 30.

7. A device according to claim 1 wherein said lower gas permeable film 120 including sections which project into said cell culture compartment.

8 A cell culture device according to claim 1 wherein at least the perimeter of said gas film support 130 comprises the lowest point of said gas film support 130 and resides on a substantially horizontal plane such that when said cell culture device is placed on a flat surface the entire surface of said, lowest portion of said gas film support 130 makes contact with the flat surface.

9. A cell culture device according to claim 1 wherein a plurality of cell culture compartments 40 are present.

10. A device according to claim 9, wherein a plurality of basal medium compartments 30 are present.

11. A cell culture device comprising a container having

a) a cell culture compartment comprised of a lower gas permeable film 120 and an upper sheet 20 selectively permeable to compounds of selected sizes and by appropriate means adapted to allow a culture medium 50 to reside between said upper sheet 20 and said lower gas permeable film 120;

b) a basal medium compartment 30, at least a portion of which is above said upper sheet 20, and adapted to allow a basal medium 60 to reside upon said upper sheet 20 and a gas to reside above and in contact with said basal medium 60;

c) an access port 70 to said cell culture compartment 40;

d) an access port 90 to said basal medium compartment 30;

e) a gas film support 130 below and in partial contact with said gas permeable film 120 whereby the majority of said gas permeable film 120 is held in a substantially horizontal position such that suspension cells can distribute across the horizontal portion of said gas permeable film 120 and gas transfer into and out of said cell culture compartment 40 is not substantially impaired; and

f) a gas exchange compartment adapted by appropriate means to provide gaseous communication between said gas of said basal medium compartment 30 and the underside of said gas permeable film 120.

12. A device according to claim 11 wherein said lower gas permeable film 120 including sections which project into said cell culture compartment.

13. A device according to claim 11 wherein said gas exchange compartment is adapted to prevent said basal medium 60 from entering said gas exchange compartment.

14. A device according to claim 11 wherein said gas exchange compartment has an access port 210 whereby condensation can be removed and gas other than that of said basal medium compartment can communicate with said the underside of said gas film.

15. A cell culture device according to claim 11 wherein a plurality of cell culture compartments 40 are present.

16. A device according to claim 15, wherein a plurality of said basal medium compartments 30 are present.

17. A cell culture device comprising a container having

a) a cell culture compartment defined by a first gas permeable film 120 and an upper sheet 20 selectively permeable to compounds of selected sizes and separated by appropriate means to allow a culture medium 50 to reside between said upper sheet 20 and said first gas permeable film 120;

b) a basal medium compartment 30, at least a portion of which is above said upper sheet 20, and adapted by appropriate means to allow a basal medium 60 to reside in contact with said upper sheet 20;

c) an access port 70 to said cell culture compartment;

d) an access port 90 to said basal medium compartment;

e) a gas film support 130 below and in partial contact with said first gas permeable film 120 whereby the majority of said gas permeable film 120 is held in a substantially horizontal position such that suspension cells can distribute across the horizontal portion of said gas permeable film 120 and gas transfer into and out of said cell culture compartment 40 is not substantially impaired;

f) a second gas permeable film 230 disposed in a horizontal position below said first gas permeable film 120;

g) one separating means between said first gas permeable film 120 and said second gas permeable film 130 to form an oxygen tension control compartment 220 adapted to contain fluid; and

e) an access port to said oxygen tension control compartment 250 whereby liquid can be added or removed to control the rate of gas transport.

18. A device according to claim 17 wherein said first gas permeable film 120 including sections which project into said cell culture compartment.

19. A cell culture device according to claim 17 wherein a plurality of cell culture compartments 40 are present.

20. A device according to claim 19, wherein a plurality of said basal medium compartments 30 are present.

21. A method of culturing cells comprising the steps:

a) forming a cell culture compartment 40, said cell culture compartment comprised of a lower gas permeable film 120 and an upper sheet 20 permeable to a selected class of compounds;

b) maintaining said lower gas permeable film 120 in a substantially horizontal position;

c) placing a basal medium 60 in a second compartment above [said upper sheet 20], said basal medium 60 in contact with the upper surface of said upper sheet 20;

d) placing the cells and a cell culture medium 50 in said cell culture compartment 40, said cell culture medium 50 in contact with said upper sheet 20;

e) maintaining cells at a selected temperature; and

f) allowing gas exchange through said lower gas permeable film 120 whereby cells are allowed to proliferate upon the surface of said lower gas permeable film 120.

22. A method according to claim 21 wherein said lower gas permeable film 120 including sections which project into said cell culture compartment 40.

23. A method according to claim 21 wherein a plurality of said cell culture compartments 40 are formed.

24. A method of culturing cells comprising the steps:

a) forming a cell culture compartment 40, said cell culture compartment 40 comprised of a gas permeable film 120 disposed below an upper sheet 20 permeable to a selected class of compounds;

b) forming a gas film support 130, said gas film support 130 disposed below said gas permeable film 120 and adapted to hold said gas permeable film 120 in a substantially horizontal position while allowing gas to contact the underside of said gas permeable film 120;

c) placing a basal medium 60 in a basal medium compartment 30 [above said upper sheet 20], said basal medium 60 in contact with the upper surface of said upper sheet 20;

d) placing the cells and a cell culture medium 50 in said cell culture compartment 40, said cell culture medium 50 in contact with said upper sheet 20; and

e) maintaining cells at a selected temperature; and

f) allowing gas exchange through said lower gas permeable film 120 whereby cells are allowed to proliferate upon the surface of said lower gas permeable film 120.

25. A method according to claim 24 wherein said lower gas permeable film 120 including sections which project into said cell culture compartment 40.

26. The method of claim 24 wherein the average distance between said upper sheet 20 and the substantially horizontal portion of said lower gas permeable film 120 does not exceed 15 millimeters.

27. A method according to claim 24 wherein a plurality of said cell culture compartments 40 are formed.

28. A method of culturing cells comprising the steps:

a) forming a cell culture compartment 40, said cell culture compartment 40 comprised of a lower gas permeable, liquid permeable film 120 and an upper sheet 20 permeable to a selected class of compounds;

b) placing a basal medium 60 in a second compartment [above said upper sheet 20], said basal medium 60 in contact with the upper surface of said upper sheet 20;

c) placing the cells and a cell culture medium 50 in said cell culture compartment 40, said cell culture medium 50 in contact with said upper sheet 20;

d) maintaining cells at an appropriate temperature; and

e) allowing gas exchange through said lower gas permeable, liquid permeable film 120.

29. A method according to claim 28 wherein said lower gas permeable, liquid permeable film 120 including sections which project into said cell culture compartment 40.

30. A method according to claim 28 wherein a plurality of said cell culture compartments 40 are formed.

31. A method of culturing cells comprising the steps:

a) forming a cell culture compartment 40, said cell culture compartment 40 comprised of a lower first gas permeable film 120 and an upper sheet 20 permeable to a selected class of compounds;

b) placing said cell culture compartment 40 upon an oxygen control compartment 220 comprised of an upper support mesh 270 and a bottom second gas permeable film 230, said support mesh 270 maintaining said lower first gas permeable film 120 in a substantially horizontal position;

c) placing a basal medium 60 in a basal medium compartment 30 [above said upper sheet 20], said basal medium 60 in contact with the upper surface of said upper sheet 20;

d) placing the cells and a cell culture medium 50 in said cell culture compartment 30, said cell culture medium 50 in contact with said upper sheet 20;

e) placing a selected volume of a liquid 260 into said oxygen control compartment 220;

f) maintaining cells at an appropriate temperature;

g) allowing oxygen and carbon dioxide to diffuse through said lower gas permeable film 120 by way of said liquid 260 and said bottom second gas permeable film 230 of said oxygen control compartment 220 whereby cells are allowed to proliferate upon the surface of said lower first gas permeable film 120, evaporation of said cell culture medium 50 is limited, and oxygen tension within said cell culture compartment 40 can be varied by adding or removing said liquid 260 from said oxygen control compartment 220.

32. A method according to claim 31 wherein said lower first gas permeable film 120 including sections which project into said cell culture compartment 40.

33. The method of claim 31 wherein said oxygen control compartment 220 is open to said basal medium 60 and adapted by appropriate means to allow a selected volume of said basal medium 60 to reside in said oxygen control compartment 220.

34. A method according to claim 31 wherein a plurality of said cell culture compartments 40 are formed.

35. A method of culturing cells comprising the steps:

a) forming a cell culture compartment 40, said cell culture compartment 40 comprised of a lower gas permeable film 120 and an upper sheet 20 permeable to a selected class of compounds;

b) forming a basal medium compartment 30, said basal medium compartment 30 adapted to hold a basal medium 60 in contact with said upper sheet 20 of said cell culture compartment 40;

c) forming an access port 70 to said cell culture compartment 40;

b) maintaining said lower gas permeable film 120 in a substantially horizontal position;

c) placing said basal medium 60 into said basal medium compartment 30;

d) placing the cells and a cell culture medium 50 in said cell culture compartment 40, said cell culture medium 60 in contact with the underside of said upper sheet 120;

e) allowing a pressure equilibrium to be established across said upper sheet 20 of said cell culture compartment 40;

e) maintaining cells at a selected temperature; and

f) allowing gas exchange through said lower gas permeable film 120 whereby cells are allowed to proliferate upon the surface of said lower gas permeable film 120.

36. A method according to claim 35 wherein a plurality of said cell culture compartments 40 are formed.

37. A method according to claim 35 wherein:

a) the entrance of said access port 70 to said cell culture compartment 40 resides at a height equal to or greater than the upper surface of said basal medium 60;

38. A method according to claim 35 wherein said lower gas permeable film 120 including sections which project into said cell culture compartment 40.

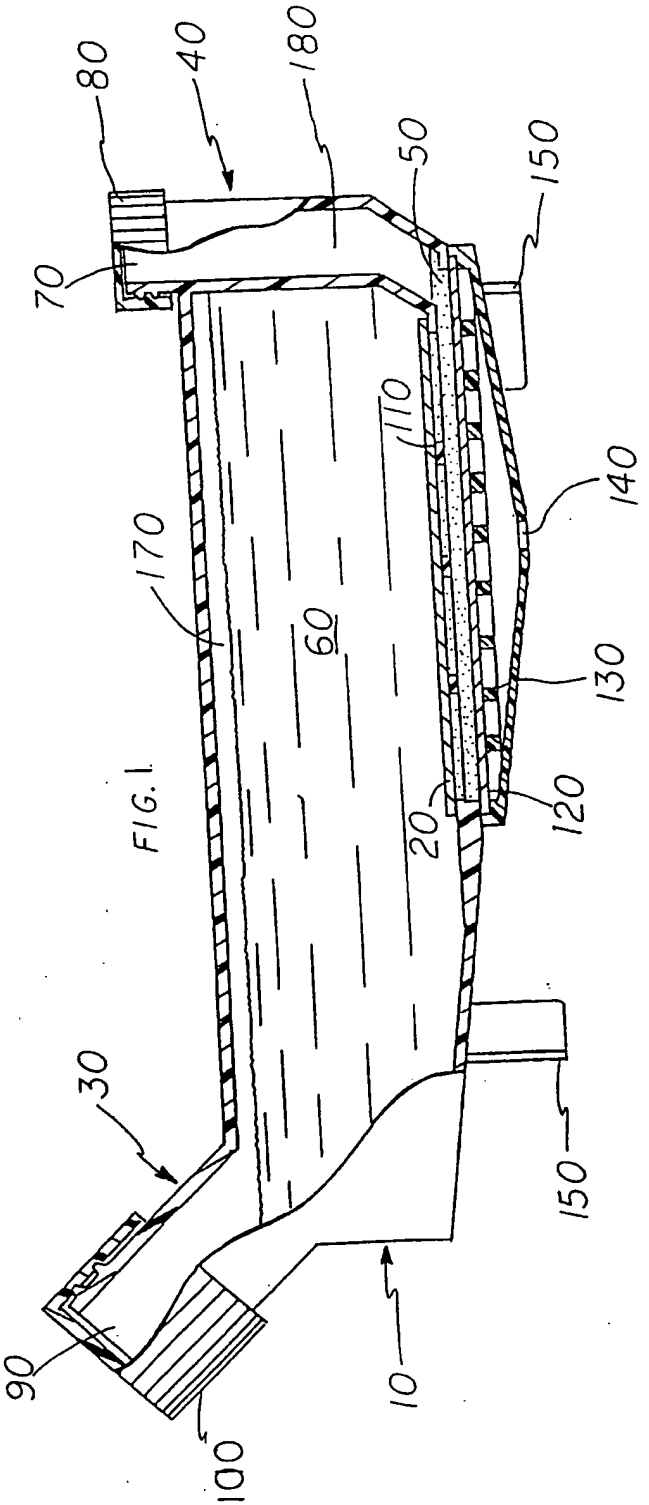

FIG. 1

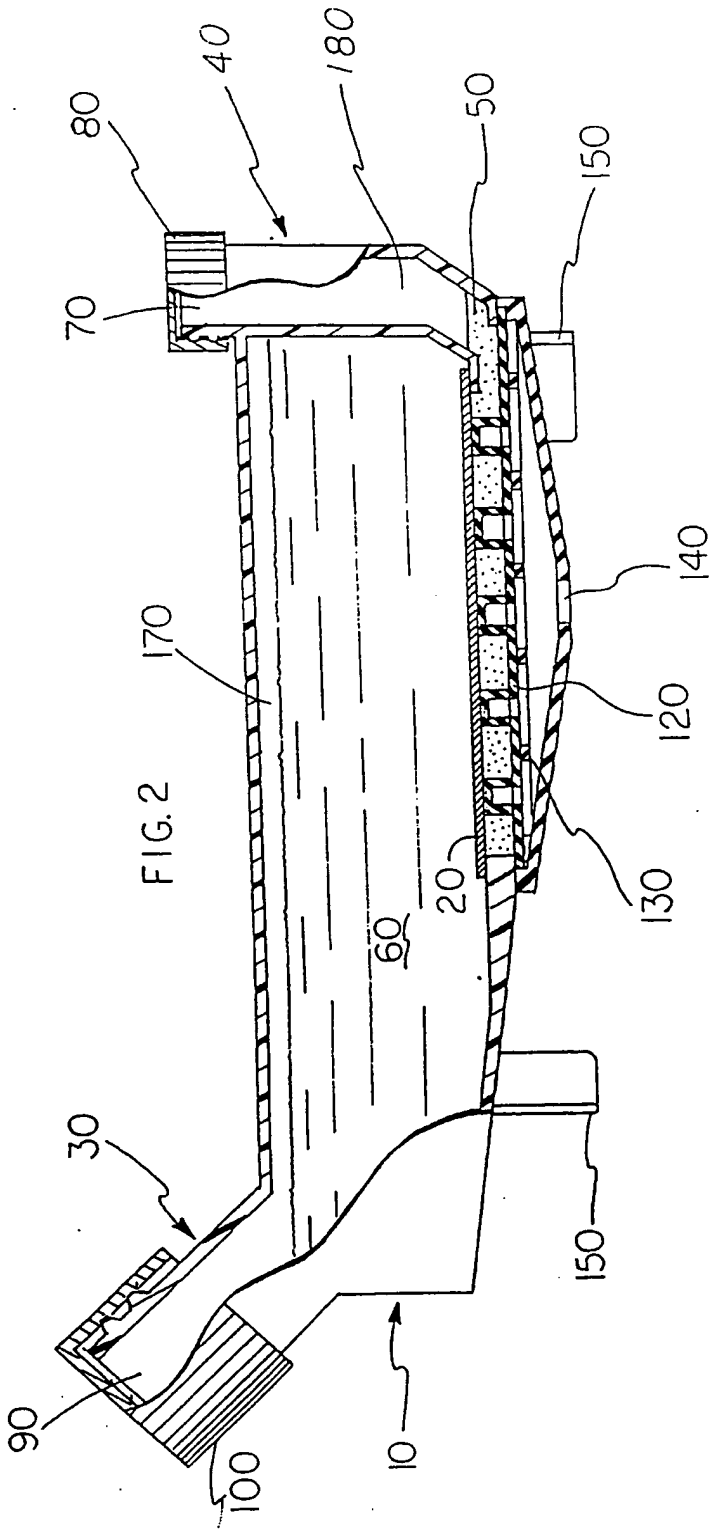

FIG. 2

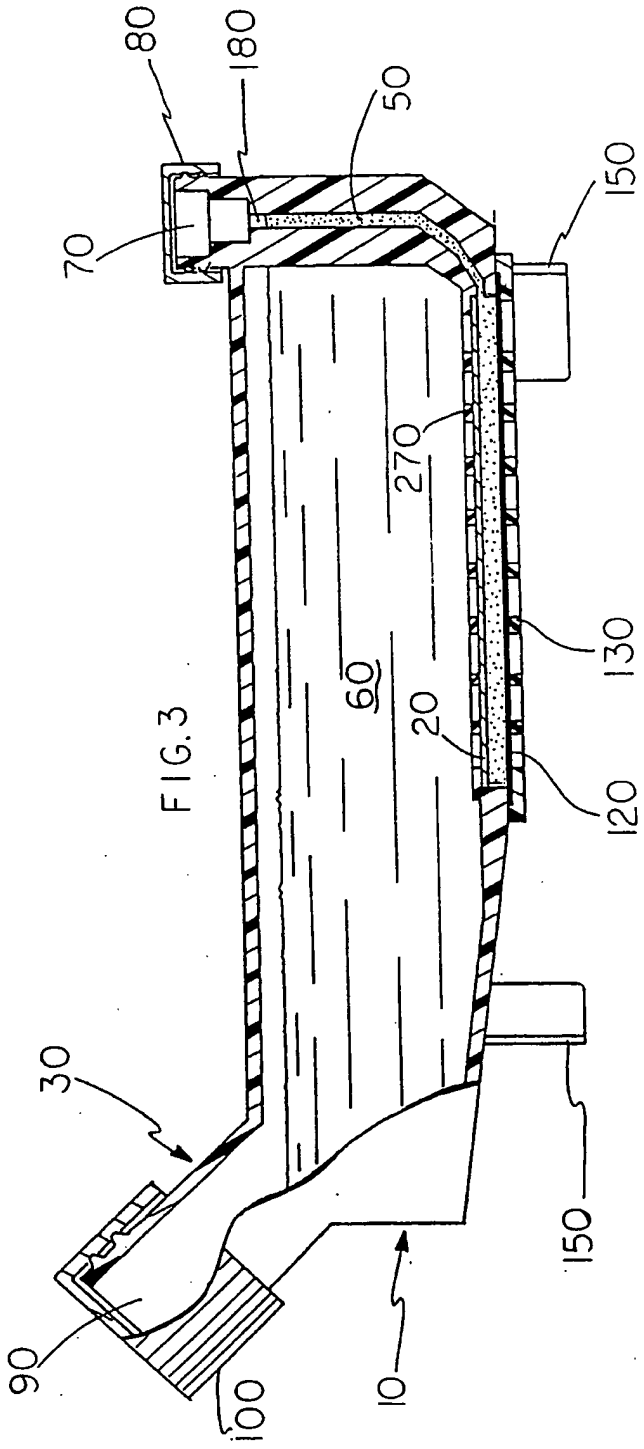

FIG.3

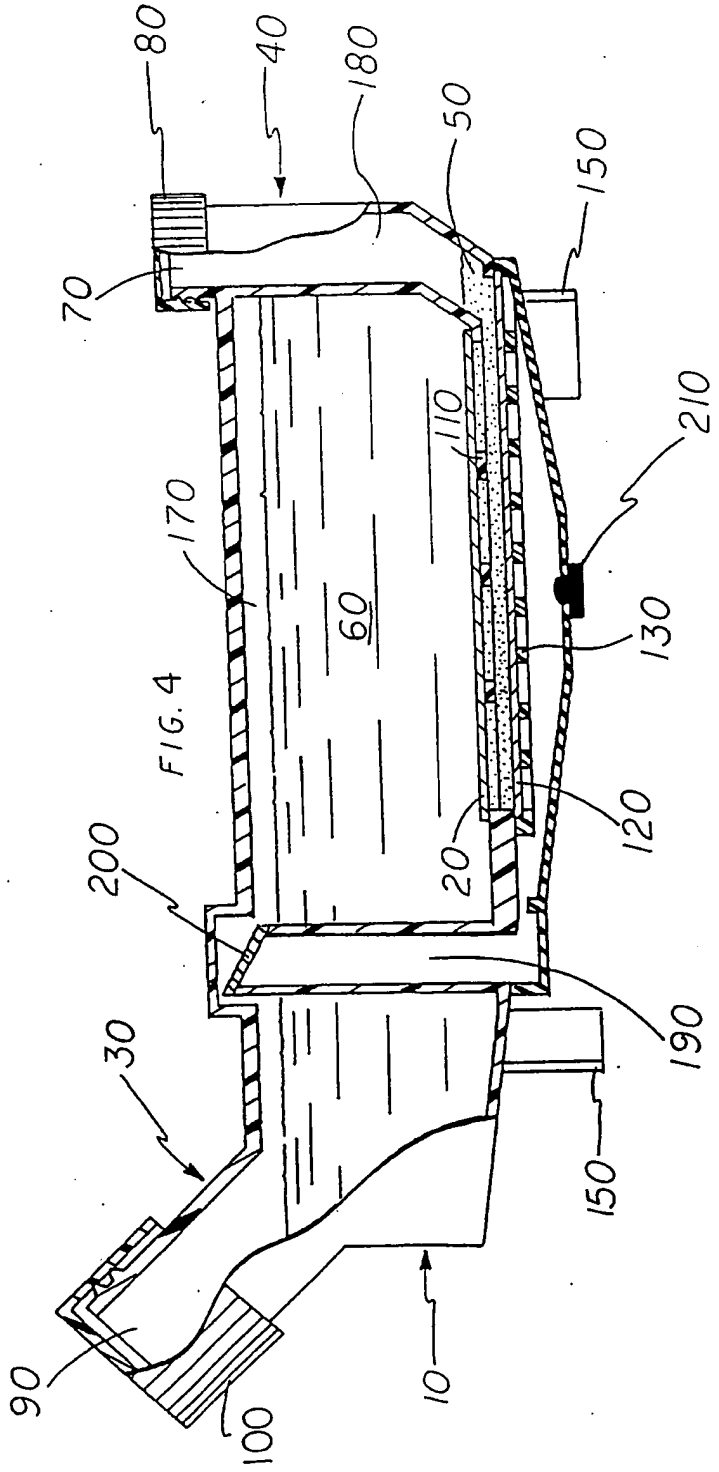

FIG. 4

SUBSTITUTE SHEET (RULE 26)

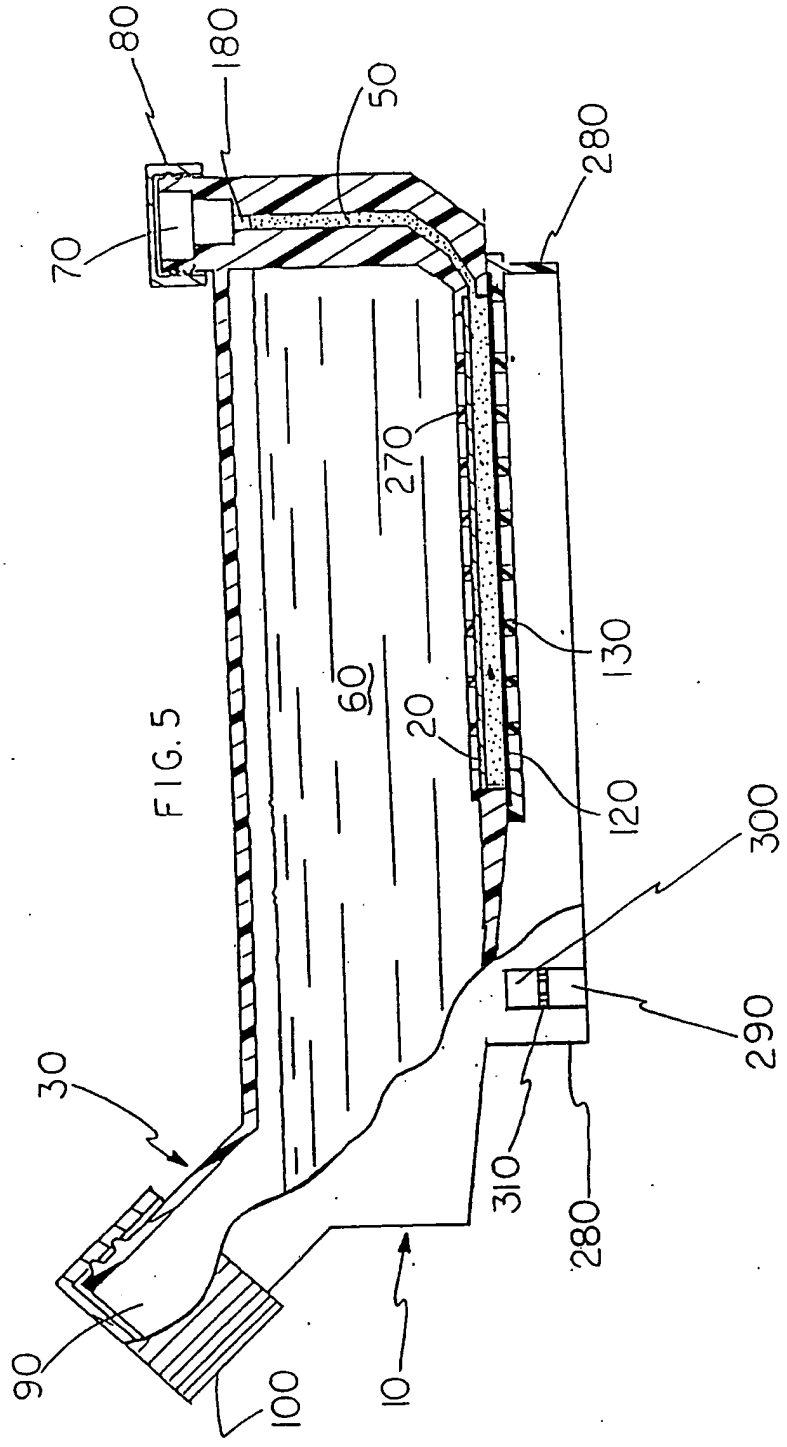

FIG. 5

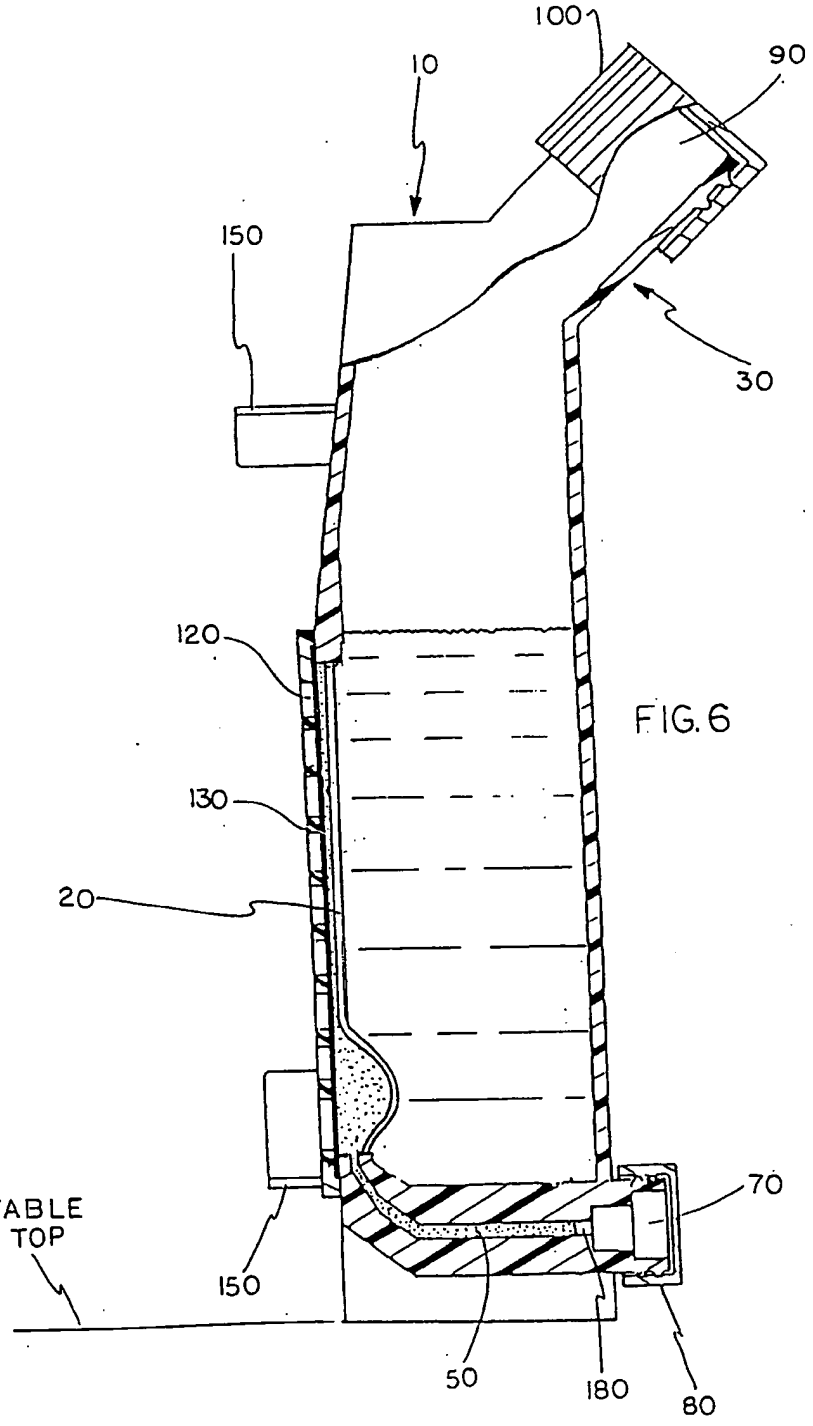

FIG. 6

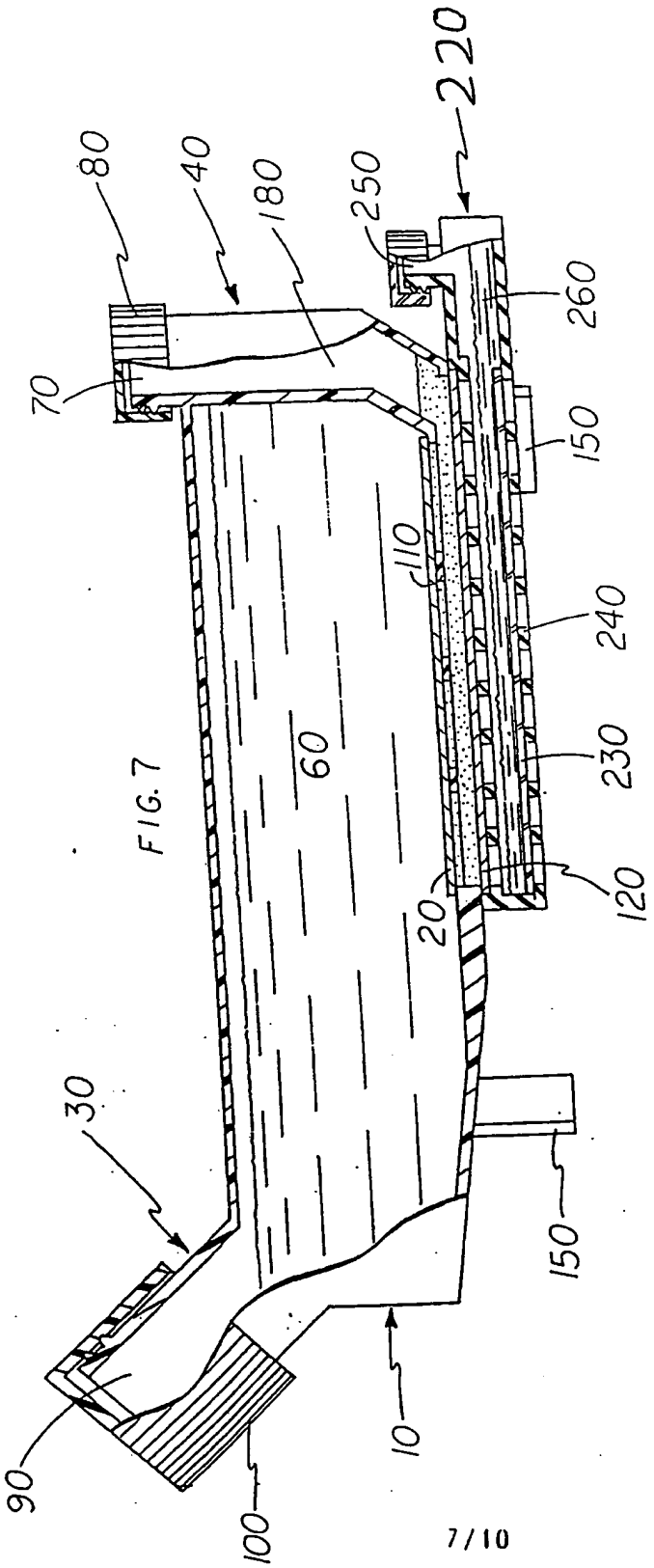

FIG. 7

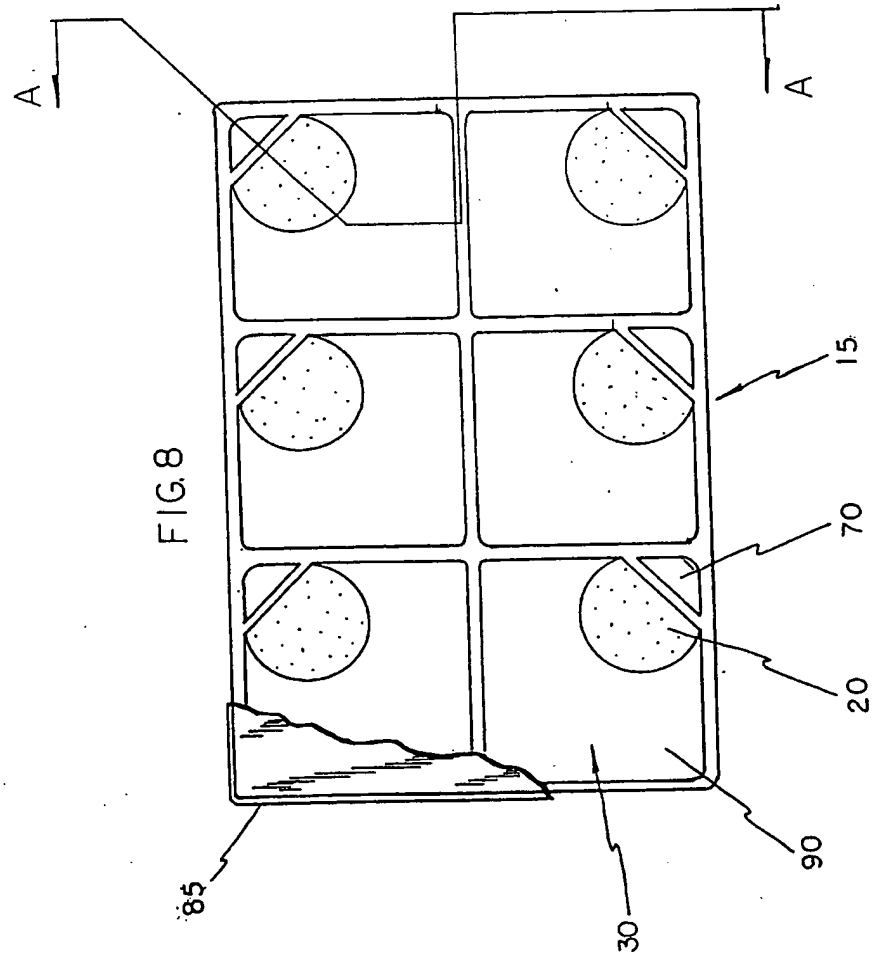

FIG.8

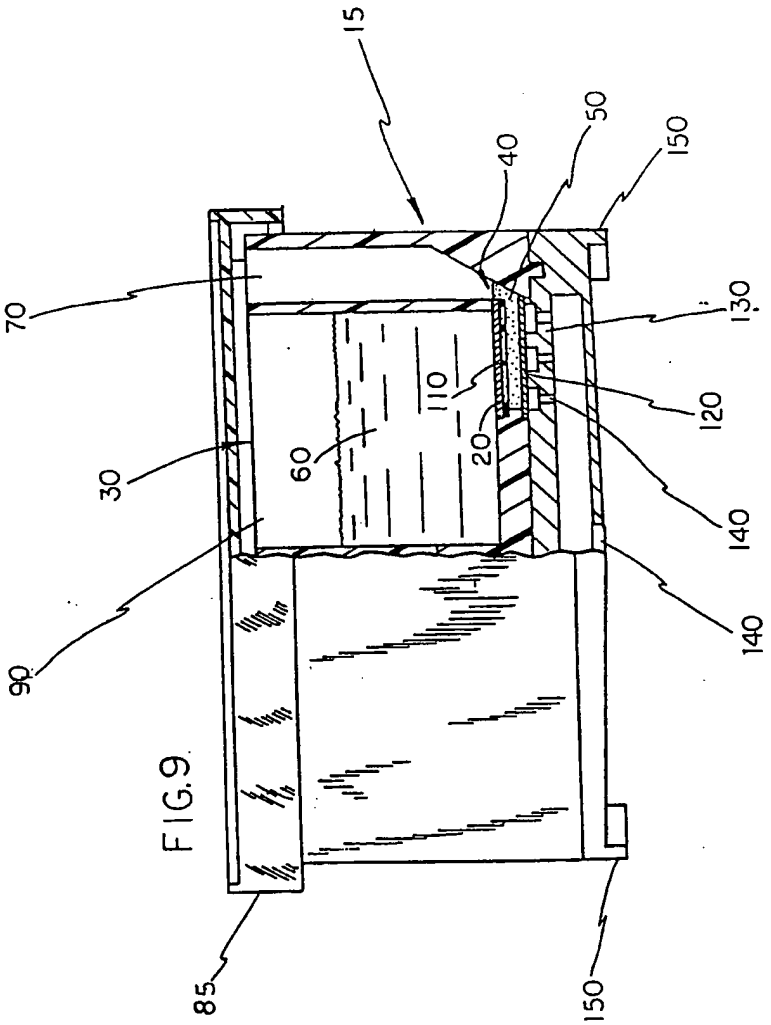

FIG.9

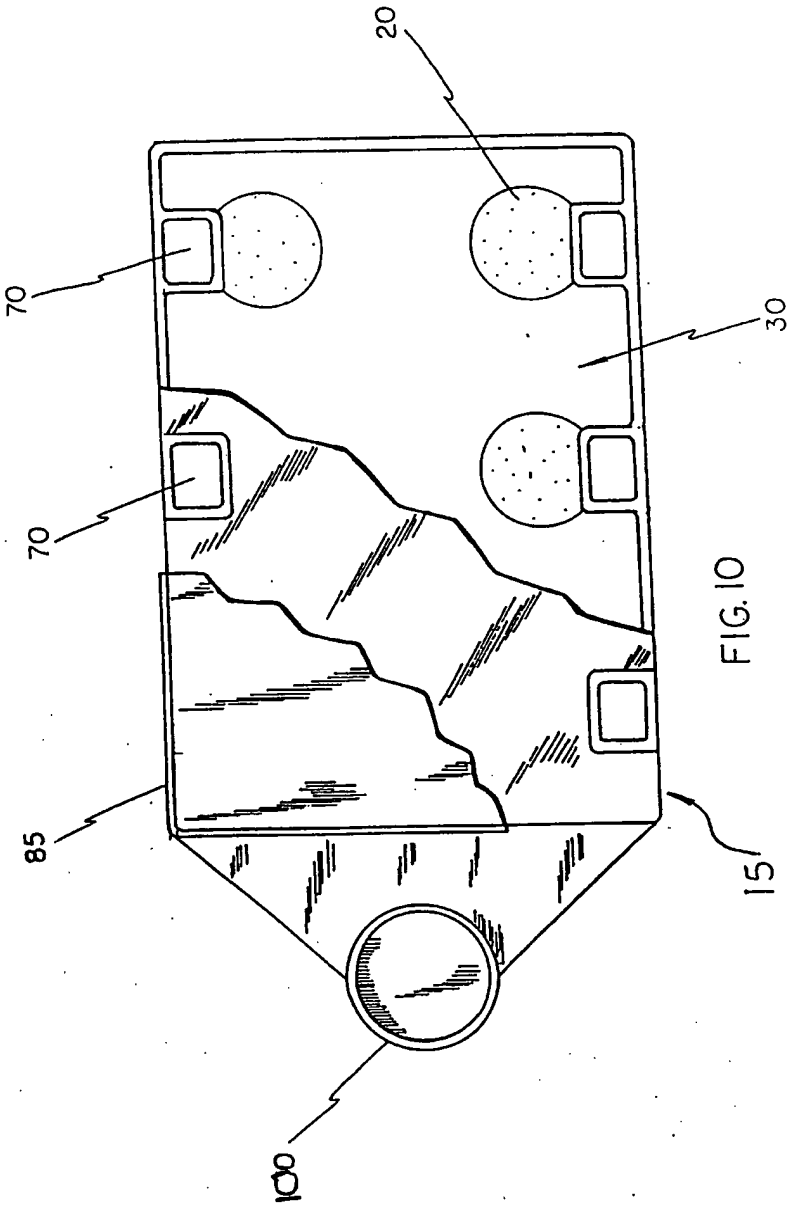

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08202

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/02; C12M 3/06
US CL :435/240.241, 284
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.1, 240.2, 240.23, 240.241, 240.25, 284-286, 296-301, 809

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US, A, 4,661,455 (HUBBARD) 28 April 1987, see entire document.	1-6 ------ 9, 10, 21-23, 26, 27, 35, 37
Y	US, A, 4,748,124 (VOGLER) 31 May 1988, see entire document.	1-5, 9, 10, 21-23, 26, 27, 35, 37
X ----- Y	US, A, 4,937,196 (WRASIDLO ET AL.) 26 June 1990, see entire document.	1-5 ------ 9, 10, 21-23, 26, 27, 35, 37

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 AUGUST 1995	Date of mailing of the international search report 04 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer WILLIAM H. BEISNER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08202

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 0,155,237 (KATINGER ET AL.) 18 September 1985, see entire document.	1-5, 9, 10, 21-23, 26-28, 30, 35, 37
Y	GIBCO BRL CATALOGUE & REFERENCE GUIDE, published 1992 by Life Technologies, Inc., see pages 6-8 and 78, see especially page 78.	21-23, 26-28, 30, 35, 37